Genetic Diversity and Adaptation of Date Palm (*Phoenix dactylifera* L.)

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University of Helsinki
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(*Phoenix dactylifera* L.)

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Academic Dissertation

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I dedicate this book to the souls of "umi" Mariam, "Aboi" Mohamed and Mahassin. May God's Grace Be With You.

Cover illustration: Date palm tree in its habitat with undetached offshoots and seedlings (Background picture); Date palm trees endangered by sand dunes and desertification (front picture). Site: Gaab-Elsawani, Northern State, Sudan. Photos by Idris Gamal

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LIST OF ORIGINAL PAPERS

The thesis is based on the following papers, which will be referred to in the text in Arabic numerals (1-4). The published papers are reprinted with the permission from the publishers.

1. **Elshibli S**, and Korpelainen H (2008). Microsatellite markers reveal high genetic diversity in date palm (*Phoenix dactylifera* L.) germplasm from Sudan. Genetica 134: 251-260.

2. **Elshibli S**, and Korpelainen H (2009). Excess heterozygosity and scarce genetic differentiation in the populations of *Phoenix dactylifera* L.: human impact or ecological determinants. Plant Genetic Resources: Characterization and Utilization 7: 95–104.

3. **Elshibli S**, and Korpelainen H (2009). Biodiversity of date palms (*Phoenix dactylifera* L.) in Sudan: chemical, morphological and DNA polymorphisms of selected cultivars. Plant Genetic Resources: Characterization and Utilization 7: 194–203.

4. **Elshibli S**, Elshibli E, and Korpelainen H. Drought adaptation of date palm (*Phoenix dactylifera* L.): morphological and physiological comparison of selected cultivars. A manuscript submitted for publication.

CONTRIBUTION OF THE AUTHORS

Papers 1, 2 and 3

Sakina Elshibli has been responsible for the original ideas, designing the experimental work, collecting plant materials in the field, performing molecular work in the laboratory, carrying out greenhouse work as well as analysing the data. Sakina Elshibli has also prepared the first drafts of the manuscripts and she has been responsible for writing the final versions in collaboration with Helena Korpelainen. Sakina Elshibli also conducted all correspondence concerning the publications. Helena Korpelainen, the supervisor of Sakina Elshibli, provided advice for data collection in the field, laboratory methods during molecular work and for data analysis.

Paper 4

Sakina Elshibli has been responsible for the original ideas, designing the experimental work, collecting plant materials in the field and carrying out the greenhouse experiment. Elshibli Elshibli collaborated with Sakina Elshibli in the data management and analysis as well as in applying the data to the photosynthetic gas exchange model. Sakina Elshibli has also prepared the first draft of the manuscript and she has been responsible for writing the final version in collaboration with Helena Korpelainen. Sakina Elshibli also conducted all correspondence concerning the publication process.

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ABSTRACT

Acquiring sufficient information on the genetic variation, genetic differentiation, and the ecological and genetic relationships among individuals and populations are essential for establishing guidelines on conservation and utilization of the genetic resources of a species, and more particularly when biotic and abiotic stresses are considered. The aim of this study was to assess the extent and pattern of genetic variation in date palm *(Phoenix dacttylifera L)* cultivars; the genetic diversity and structure in its populations occurring over geographical ranges; the variation in economically and botanically important traits of it and the variation in its drought adaptive traits, in conservation and utilization context.

In this study, the genetic diversity and relationships among selected cultivars from Sudan and Morocco were assessed using microsatellite markers. Microsatellite markers were also used to investigate the genetic diversity within and among populations collected from different geographic locations in Sudan. In a separate investigation, fruits of cultivars selected from Sudan, involved morphological and chemical characterization, and morphological and DNA polymorphism of the mother trees were also investigated. Morphological and photosynthetic adjustments to water stress were studied in the five most important date palm cultivars in Sudan, namely, Gondaila, Barakawi, Bitamoda, Khateeb and Laggai; and the mechanism enhancing photosynthetic gas exchange in date palm under water stress was also investigated.

Results showed a significant ($p < 0.001$, t-test) differentiation between Sudan and Morocco groups of cultivars. However, the major feature of all tested cultivars was the complete lack of clustering and the absence of cultivars representing specific clones. The results indicated high genetic as well as compositional and morphological diversity among cultivars; while, compositional and morphological traits were found to be characteristic features that strongly differentiate cultivars as well as phenotypes. High genetic diversity was observed also in different populations. Slight but significant ($p < 0.01$, AMOVA) divergence was observed for soft and dry types; however, the genetic

divergence among populations was relatively weak. The results showed a complex genetic relationships between some of the tested populations especially when isolation by distance was considered. The results of the study also revealed that date palm cultivars and phenotypes possess specific direct or interaction effects due to water availability on a range of morphological and physiological traits. Soft and dry phenotypes responded differently to different levels of water stress, while the dry phenotype was more sensitive and conservative. The results indicated that date palm has high fixation capacity to photosynthetic CO_2 supply with interaction effect to water availability, which can be considered as advantageous when coping with stresses that may arise with climate change.

In conclusion, although a large amount of diversity exists among date palm germplasm, the findings in this study show that the role of biological nature of the tree, isolation by distance and environmental effects on structuring date palm genome was highly influenced by human impacts. Identity of date palm cultivars as developed and manipulated by date palm growers, in the absence of scientific breeding programmes, may continue to mainly depend on tree morphology and fruit characters. The pattern of genetic differentiation may cover specific morphological and physiological traits that contribute to adaptive mechanisms in each phenotype. These traits can be considered for further studies related to drought adaptation in date palm.

Keywords: *Genetic diversity, microsatellite markers, Phoenix dactylifera L., populations, morphological traits, physiological traits, photosynthesis, water stress*

أظهرت النتائج وجود درجة عالية من التنوع الوراثي في كل الأصناف المختبرة و تمايز معنوي احصائيا بين أصناف السودان و أصناف المغرب، و مع ذلك فإن السمة الرئيسية لجميع الأصناف المختبرة هي الإنعدام التام لتمييز صنف منفردا عن الآخر، مما يدل على أن العمليات الفلاحية لنخيل التمر تشمل خلط أشجار بذرية مع شتول ناتجة عن التكاثر الخضري، و لذلك يظل التعرف على الأصناف المختلفة قاصرا على الصفات الظاهرية للأمهات و الثمار و الكيمائية و الظاهرية للثمار. أظهرت النتائج ايضا وجود تنوع وراثي كبير داخل العشائر المختلفة في جميع المواقع الجغرافية التي تم جمع العينات منها. كما لوحظ وجود تباين معنوي إحصائيا بين المجموعات التي صنفت كأصناف جافة و المجموعات التي صنفت كأصناف رطبة وهذا قد يدل على وجود صفات وراثية خاصة لكل مجموعة. أظهرت النتائج وجود علاقات معقدة بين بعض العشائر بالرغم من وجود مسافات جغرافية بينهما تفوق 2000 كلم. و يرجع هذا التعقيد للدور الذي يلعبه الإنسان أثناء عمليات تبادل عوامل الإنتشار مثل الشتول، حبوب اللقاح، الثمار و بالتالي البذور.
نتائج الدراسة كشفت أيضا عن أن نقص طول النبات، شيخوخة مبكرة للاوراق و نقص مجموع عدد الوريقات من الآثار الناتجة عن محاولة تحمل الاجهاد المائى. كشفت الدراسة أيضا عن وجود إختلافات معنوية إحصائيا بين الأصناف الرطبة و الأصناف الجافة في عدد الوريقات ، التمثيل الضوئي للوريقة ودرجة تثبيت العرض الضوئي لثانى اوكسيد الكربون Co_2 .

خلصت الدراسة الى انه بالرغم من وجود تنوع وراثي كبير في كل اللأصناف إلا أن الدلائل التى تم استخدامها غير قادرة على تمييز صنف عن الآخر. و تتفق هذه النتائج مع نتائج لباحثين استخدموا انواع اخرى من الدلائل الجزيئية. و في حال عدم وجود تربية علمية لأصناف نخيل مختارة يظل تصنيف هذه الأصناف قاصرا على الصفات الظاهرية و الإنتاجية. نمط التمايز الجيني عند بعض الأصناف الجافة و الرطبة و الذي يزداد عند التعرض للإجهاد غير الحيوي (مثلا لإختبارات مائية مختلفة) يدل على وجود خصوصية تكيف بيئية لكل مجموعة يمكن دراستها مستقبلا للإستفادة الممكنة في برامج التربية.

ملخص

بسم الله الرحمن الرحيم

وبه الإعانة بدءً وختما

وصلى الله على سيدنا محمد ذاتا ووصفا واسما

التنوع الوراثي و التكيف البيئي لنخيل التمر

تعتبر شجرة نخيل التمر أهم شجرة فاكهة في شمال السودان حيث أنها زرعت في السودان قبل 3000 سنة، و من ثم امتدت الى ارجاء السودان المختلفة، و يقدر عدد الأصناف الموجودة في السودان بحوالى 400 صنف. بجانب أهميتها كشجرة فاكهة فان أشجار النخيل تلعب دورا فاعلا في توفير المناخ المناسب لزراعة الخضروات والعلف بجانب الاستخدامات الاقتصادية و الاجتماعية الاخرى. بالرغم من القيمة الغذائية، الآقتصادية، الآجتماعية والدينية العالية لزراعة النخيل في السودان إلا أن الإهتمام بدراسة تنوعه الوراثي يعتبر الأقل حيث أنه لاتوجد دراسة سابقة لذلك. الحصول على معلومات كافية عن الأختلاف الجيني، التميز الوراثي والعلاقات البيئية والوراثية بين الأفراد و العشائر ضروري لوضع مبادئ توجيهية بشأن الحفظ و الأستفادة من الموارد الوراثية للصنف النباتى. و تزيد أهمية هذه المعلومات و ضرورة الأستفادة منها عندما نضع في الآعتبار الاثار السالبة الناتجة عن الآجهاد الحيوي و غير الحيوي والناتج بعضها عن آثار التغير المناخي.

تعتمد هذه الدراسة على أربعة محاور بهدف تحقيق الاتى:- اولا تقييم التنوع الجينى و التمييز الوراثي بين أصناف نخيل مختارة من السودان و المغرب و ذلك باستخدام الدلائل الجزيئية ، ثانيا تقييم التنوع و التمييز الوراثي داخل و فيما بين عشائر تم جمعها من مواقع جغرافية مختلفة من السودان شملت مواقع في الولاية الشمالية، ولاية نهر النيل، ولاية البحر الأحمر(سلوم) و ولاية شمال كردفان حيث واحات البشيري و أبو قايدة. ثالثا توصيف مورفولوجي و كيميائي لأصناف تم اختيارها من مشتل بساتين نوري بالسودان، وقد شمل التوصيف صفات الثمار، البذور و الأمهات، بينما كان التركيز في التوصيف الكيميائي للثمار على محتوى ذات الأصناف لأنواع مختلفة من السكر و درجة الحموضة. رابعا دراسة و تقييم آثار الإجهاد المائي و الجفاف البيئ على الصفات المورفولوجية و الفسيلوجية لأصناف شملت قنديلة ، بتمودا، بركاوى ، مشرق ود خطيب و مشرق ود لقاي.

1. INTRODUCTION

1.1. Genetic diversity of tree species

The main biological features of tree species include the characteristic long life cycles and the lack of mobility which necessitate a need to withstand exposure to large fluctuation in their environmental conditions. Consequently, the need for the adaptability of trees is extremely high compared to other organisms. To fulfill these demands, tree species need to maintain large amounts of genetic variation for the preservation of adaptability and survival to subsequent generations (Müller-Starck and Gregorius 1968). Thereby, trees have developed natural mechanisms to maintain high levels of genetic variation within species. These include high rates of outcrossing and the dispersal of pollen and seeds over wide areas as well as different modes of reproduction. These mechanisms, combined with changing environments, have contributed to the evolution of forest and tree species into more genetically diverse organisms compared to herbaceous species (Hamrick et al. 1992, Hamrick and Godt 1996). Genetic variation within a species is needed to ensure today and future adaptability of species as well as their continued evolution. Variation is also necessary to maintain options and potential for genetic improvement to meet changing end-use requirements and dynamically evolving environmental conditions, allowing for domestication and breeding programmes. Obtaining sufficient information on the extent and pattern of genetic diversity, population differentiation occurring over geographical ranges, and understanding of the ecological and genetic relationships among individuals and populations are essential for establishing guidelines on utilization and conservation of the genetic resources of a species, especially when biotic and abiotic stresses are considered (Bradshaw 1975, Bates 1985, Namkoong 1989, Frankel et al. 1995, Virchow 1999).

1.2. Date palm, *Phoenix dactylifera* L.

Date palm (*Phoenix dactylifera* L.) is a multipurpose tree, with a long history of cultivation and utilization in north Africa and Middle East for at least 5 000 years. Date palm is a diploid (2n=36), perennial, monocotyledonous plant. Being a dioecious species in character, date palm has separate male and female individuals. Female trees are cultivated mainly for their nutritive fruits. Although the average economic life of a date palm tree is estimated to be up to 50 years, the tree may stay productive up to 150 years (Chao and Krueger 2007). On the other hand, the average pollen bearing capacity of a good male palm would be sufficient to pollinate 50 female palms, determined by both the number of flowers and the pollen quantity per flower (Zaid and de Wet 2002a). However, pollen of different males has been found to have different effects on the size of fruits and seeds (xenia) as well as time of fruit ripening (metaxenia).

The importance of date palm culture for its high nutritive, economic and social values is well recognised, especially in arid and semi-arid areas where it plays an important role in affecting microclimate in a way that enhances the production of other agricultural crops. Worldwide production, utilisation and industrialisation of dates are increasing continuously (Botes and Zaid 2002); the world production of dates has increased from 1.8 million tons in 1961 to 5.4 million tons in 2001, with an annual expansion of about 5%.

The geographic distribution of date palm covers a wide range of environmental conditions. For instance, it grows and flourishes from 392 m below to 1 500 m above sea level with an altitude range of 1 892 m (Zaid and de Wet 2002 b). Besides the wide range of geographic distribution, also the biological nature of date palm is expected to affect the genetic structure and culture of date palm worldwide. In many production areas in the world, serious stresses resulting in considerable losses have been reported on date palm culture. These stresses include, e.g., diseases, drought, desertification and floods (Baaziz et al. 2000, Zaid et al. 2002c; United Nations Environment Programme 2007). Millions of date palm trees were lost within one decade in North Africa due to such natural disasters (Baaziz et al. 2000). These facts suggest that large numbers of traditional cultivars in many countries may be diminishing, and it has been suggested that urgent conservation efforts are needed worldwide (Chao and Krueger 2007).

1.2.1. Development of date palm cultivars

Thousands of date palm cultivars exist in different growing countries. These cultivars have been developed by continuous selection performed by date palm growers all over the world mainly to improve crop yield and quality. Date palm counts as high as 5000 cultivars all around the world (Jaradat and Zaid 2004). Based on botanical description, there are about 400 cultivars in Iran, 370 in Irag, 250 in Tunisia, 244 in Morocco (Zaid and de Wet 2002d) and 400 in Sudan (Osman 1984) as well as many additional varieties in the other major date palm growing countries. These cultivars are commonly identified by a wide range of morphological features describing trees and fruits (Nixon 1950, Zaid and de Wet 2002d, Elhoumaizi et al. 2002, Osman 2002). It is confusing that sometimes the cultivars are described based on fruit characteristics or other features or both; also the given names in different countries and regions may include the same name for different cultivars and/or different names for the same cultivar as a consequence of exchange processes (Cao and Chao 2002). It is worth to mention that there is no evidence for the majority of those cultivars (or varieties) being breeders' clones. The selection, cloning and distribution of cultivars are mainly farmers' activities and developed through thousands of years. Yet, date palm specialists have attempted to list and botanically describe those cultivars in their respective countries (Zaid and de Wet 2002d).

1.2.2. Management of date palm germplasm

Date palm can reproduce both sexually and asexually. Date palms are cloned by separating and independently establishing the offshoots produced by individual trees. This method maintains the genetic integrity of date palm cultivars. Offshoots are produced in limited numbers during a date palm's life span (Zaid and de Wet 2002e). Numbers of produced offshoots vary greatly among cultivars with the typical range being 20-30. However, not all offshoots succeed to make roots and survive (Zaid and de Wet 2002e). Seeds are breeding material with long backcrossing cycles. The first flowering of a tree takes place at the age of about 5-7 years (Baaziz et al. 2000, Zaid and de Wet 2002e). Therefore, the biological characteristics of date palm trees render it very difficult to compensate for the rapid decline of specific cultivars due to natural disasters.

Extensive effort has been made to propagate date palms through tissue culture (Tisserat 1979, Beauchesne 1982, Drira and Benpadis 1985, Beacuchesne, Zaid and Rhiss 1986, Omer et al. 1992, Al-maarri 1995, Zaid and de Wet 2002e). However, clonal propagation with tissue culture techniques still has some uncertainties concerning the true type of propagated material, especially when somatic embryogenesis and callus formation are considered. Reports on growth abnormalities in *in vitro*-derived date palm plants include a wide range of traits, like failure to flower or fruit, dwarfness, loss of chlorophyll in leaves (albino), and crop failure (Djerbi 2000, Azeqour et al. 2002, Al-Wasel 2005, Al Kaabi et al. 2005). The genetic variability of tissue culture derived date palms compared to mother plants has been assessed using different genetic markers. A wide range of variability has been reported as well (Saker et al. 2000, Azeqour et al. 2002, El-Assar et al. 2005, Saker et al. 2006). However, no links have been identified among genetic variability and the observed morphological and/or physiological disorders. Commercial micropropagation and distribution of date palms with genotypically stable cultivars have been ongoing since early 1990s (Zaid and de Wet 2002e). The demonstration of the true-to-type character for the produced plants is an important part of the quality assurance and it requires the use of specific markers that are able to distinguish cultivars.

Biochemical studies, including isozyme analyses and activity analyses of peroxidases have been used to characterize date palms in Morocco and Tunisia (Baaziz and Saaidi 1988, Baaziz 1988, Bendiab et al. 1998, Salem et al. 2001, Baaziz et al. 2000, Majourhat et al. 2002). However, isozymes appear to be of a limited use due to low levels of polymorphisms (Al-Jibouri and Adham 1990). DNA markers have also been applied as a direct approach to detect genetic variation in date palm cultivars. Randomly amplified polymorphic DNA (RAPD) fingerprints have been used to identify date palm accessions in Saudi Arabia (Al-Khalifah and Askari 2003), Egypt (Soliman et al. 2003, Adawy et al. 2006), Tunisia (Trifi et al. 2000), Morocco (Sedra et al. 1998) and Algeria (Benkhalifa 1999). Benkhalifa (1999) has stated that both morphological and RAPD markers show considerable difficulties when characterizing cultivars. On the other hand, polymorphisms of date palm cultivars from Egypt with different introduction background and California have been examined by amplified fragment length polymorphism (AFLP) markers, where discrimination between cultivars from different geographic locations was not possible (Cao and Chao 2002, El-Assar et al. 2005, Adawy et al. 2006). Microsatellite markers also have been applied to assess the genetic diversity and relationships of date palm accessions in Tunisia (Zehdi et al. 2004a). The attributes of codominance, reproducibility and high resolution have all contributed to the popularity of nuclear microsatellites as powerful tools for genetic and population analyses as well as within-species genotype identification.

1.2.3. Types and ecological distribution of date palm cultivars

Dates (date palm fruits) contain high concentrations of sugars, mainly reducing ones in the form of glucose and fructose, and sucrose as a non-reducing sugar. Dates also contain cellulose and starch. It has been found that the water and sugar contents undergo changes during date ripening (Mustafa et al. 1986, Barreveled, 1993). The development of date fruits is classified into five stages, using Arabic terms: Hababouk stage starts soon after fertilization, lasts for four to five weeks and is characterized by the loss of two unfertilized carpels, Kimri stage with two phases where fruits are not suitable for eating, and the edible stages Khalal, Rutab and Tamr (Zaid and de Wet 2002d). Each stage is characterised by specific fruit colour and texture. At the Tamr stage, fruits dry to firm consistency with a dark colour. Some types of dates, considered as soft, do not develop to the Tamr stage. The water content decreases as dates ripen. The average moisture content drops from 84% at Kimri stage to 66% at Khalal, 43% at Rutab, and down to 24% at the Tamr stage (Ahmed et al. 1995). In Sudan, the Medina and Laggai dates are mainly consumed at the Rutab stage, while the easily stored dry types, such as Gondaila and Barakawi, are mainly consumed at Tamr stage. Besides sugar content, another character that has not received much attention is the fruit acidity. Apart from their general role in metabolism, acids play an important role in the context of fruit flavour and marketing. Titratable acidity is used to test the stage of ripeness, and it serves some practical purposes in the commercial assessment of fruits (Hulme, 1970). It is known that date fruits contain a number of organic acids, such as citric, malic and oxalic acid, which are considered as contributors to flavour (Barreveled, 1993).

The classification of dates into soft, semi-dry and dry types, mainly based on the texture of the ripe fruit, is thought to be associated with the content of particular sugars and water (Cook and Furr 1953, Mustafa et al. 1986, Barreveld 1993, Zaid and de Wet, 2002d). Soft and dry types of dates exist in different producing countries (Zaid and deWet 2002b), although the ecological distribution of these types has been reported only in Sudan (Osman 1984).

Figure 1. An example of the best known cultivars in Sudan described as soft and dry types, which shows the detectable variability in fruit morphology of these cultivars. Mishrig Wad Laggai, Mishrig Wad Khateeb and Medina are considered as soft types of dates. Photo by Sakina Elshibli.

The distribution of date palm culture in Sudan follows a geographic pattern, including locations for the successful production of either soft or dry type of dates (Osman 1984). Fig. 1 shows an example of the best known cultivars in Sudan described as soft and dry types, and also shows the detectable variability in fruit morphology of these cultivars.

The dry type dominates in the area from the Egyptian border in the north of Sudan to south around Abo-Hamad (19^{o} 32 $'$ N; 33 ^{o} 21 $'$ E; Osman 1984). The geographical change from dry to soft types of dates is gradual. For example, in Sudan, there are some areas in between where the commercial production of both types is successful.

1.3. Adaptation and adaptive traits

Adaptation is defined as heritable modifications in structures or functions that increase the probability of an organism surviving and reproducing in a particular environment (Kramer 1980). Adaptation of an organism to an environment depends on the possession of an optimum combination of traits that minimizes the harmful effects and maximizes the advantageous effects and can be preserved by natural selection. The structural modifications include morphological traits that help an organism to survive in its natural habitat, while functional modifications may include regulation of physiological processes that allow an organism to adapt to a certain environment.

1.3.1. Adaptation to drought

Research on plants' responses to drought and water stress is becoming increasingly important, as climate-change evolves faster than before due to increasing human impacts and industrialization. Most expected scenarios include an increase in aridity in many areas of the globe. Plant strategies to resist drought include escape, avoidance and tolerance, and may include a wide range of combinations between different response types. Plants that escape drought possess a high degree of developmental plasticity and are able to complete their life cycle or at least they reproduce successfully before the onset of severe stress; this is important in arid regions, where native annuals may have short life cycles with high photosynthetic capacities and growth rates. Plants can also resist drought conditions by avoiding tissue dehydration, by maintaining tissue water potential as high as possible, or by tolerating low tissue water potential (Levitt 1972, Jones 1980, Kramer 1980, Chaves et al. 2003).

Dehydration avoidance is common to both annuals and perennials and is associated with a variety of adaptive traits. These traits involve minimizing water loss and maximizing water uptake or maintaining a large internal storage of water (Kummerow 1980, Jones

1980, Larcher 2003, Lambers et al. 2008). Stomatal characteristics and behavior increase the ability of plants to endure drought for considerable periods of time without becoming severely dehydrated (Kramer 1980). The reduction in plant growth as a consequence of reduction in leaf area provides a mechanism for reducing the rate of water use and delaying the onset of more severe water stress. Increased access to soil water through a greater root/shoot ratio is also one of the adaptive mechanisms that maximize water uptake in response to water stress (Begg 1980).

The physiological and morphological adjustments that take place in a plant during water stress can be divided into short and long-term responses. Reduction in leaf area which can be accomplished by accelerating the rate of senescence of the physiologically older leaves and through its effect on leaf shedding is a relatively slow and irreversible response mechanism that affects more long-term adaptation than diurnal physiological behavior. Leaf movements and orientation are stress adaptation mechanisms that mainly reduce the effective leaf area and the energy load on the plant. Characteristic features of such kind of responses include reversibility and rapid recovery on the relief of stress (Begg 1980, Chaves et al. 2003).

Tolerance to low tissue water potential involves osmotic adjustment (Morgan 1984) that occurs by means of morphological or physiological modifications that reduce transpiration or increase absorption (Kramer 1980). However, stomatal adjustment to water potential and carbon dioxide recycling during photosynthesis in water stressed plants are considered as the main physiological mechanisms that have adaptive significance to water stress (Ludlow 1980, Osmond et al. 1980, Chaves et al. 2003, Izanloo et al. 2008).

The nature and extent of the response of plants to water stress are a function of the intensity and duration of the stress. These responses and their implications for field performance are controlled by the genetic characteristics of the plant and growing conditions under which it has evolved (Chaves 1991, Chaves et al. 2003, Lambers et al.

2008). Plant responses based on genetic characteristics require appropriate genetic variation that exists among and within plant species (Morgan 1984, Izanloo et al. 2008). The extremes of dry land climates mainly determine the form of the physiological adaptation and ecological requirements of plants occurring in dry land areas. Physiological control of transpiration and metabolic rates, water and food storage organs and thorns are examples of characteristics reflecting adaptation to drought. Such specialization becomes less marked as aridity becomes moderate and less acute, and the conditions for plant establishment and growth become more favourable (Turner and Kramer 1980, Ffolliott et al. 2002, Chaves et al. 2003). The production rate of a plant can be given in terms of the net assimilation rate or relative growth rate, which, in turn, may affect the dry matter content of the plant. The magnitude of the highest possible and average net assimilation rate depends mainly on the morphological and physiological constitute of the plant (Larcher 2003).

1.3.1.1. Impact of drought on photosynthesis

Low water availability is among the main environmental factors limiting photosynthesis and plant growth (Muuns 2002, Lambers et al. 2008). Generally photosynthesis is colimited by CO_2 diffusion through stomates and light-driven electron transport, which include the CO_2 supply and demand functions. The demand function is determined by the rate of processing CO_2 in the chloroplast (Lambers et al. 2008). Research reports that describe plant responses to water stress have pointed out that each of limiting CO_2 supply function (Sharkey 1990, Chaves 1991, Cronic 2000) as well as changes in metabolic demand function (Flexas and Medrano 2002) were found to take place when plants were subjected to water stress. However, there was a long-standing controversy about which of these processes is the main physiological target that limits the photosynthetic capacity of plants under water stress conditions (Flexas and Medrano 2002, Flexas et al. 2004, Flexas et al. 2006, Lawlor and Tezara 2009). On the other hand, some researchers reported that a combination of both kinds of limitations take place simultaneously in response to water stress (Pankovic et al. 1999, Lawlor 2002, Tezara et al. 2002, 2003), and considered it as a strategy allowing drought-stressed plants to escape photo damage by downregulating photosynthesis (Pankovic et al. 1999, Maroco et al. 2002). The role of genotype combined to other environmental conditions was suggested among the main factors that may lead to different kinds of responses observed in different plant species (Lawlor and Tezara 2009).

Changes in the biochemical demand function can be estimated by studying the relationship between the assimilation rate of CO_2 in leaves (A) and the CO_2 concentration in the intercellular space (Ci), as demonstrated by the biochemical gas exchange model of Farquhar et al. (1980). This model has become a common tool to estimate photosynthetic biochemical parameters of the intact leaf under a wide range of experimental conditions, including abiotic stresses (Flexas and Medrano 2002, Lawlor and Cronic 2002, Lambers et al. 2008). According to this model, for C_3 plants, carboxylation rates are limited by one of the following three processes: (1) the amount, activity and kinetics of Rubisco (Ribulose 1, 5-biphosphate carboxylase-oxygenase, (2) the RuBP (Ribulose 1, 5-biphosphate) regeneration supported by electron transport and (3) triose- phosphate utilization (TPU, Von caemmerer and Farquhar 1981, Sharkey 1985, Harley and Sharkey 1991, Harley et al. 1992).

1.3.1.2. Drought adaptation in date palm

The cultivation of date palm constitutes one of the most successful agricultural activities in arid and semi-arid regions. Adaptation of date palm to water stress is expected as it was one of the first fruit trees distributed and taken into cultivation in naturally dry lands, which are characterized by long and hot summers, low or at most no rainfall, and very low relative humidity is essential during fruit ripening period. The tree withstands large temperature fluctuations from very high up to 56 ^{o}C to few degrees below zero (Zaid and de Wet 2002). Date palm has a thick waxy cuticle, spines may constitute 1/5 - 1/4 of the pinnately compound leaf (depending on cultivar) and efficient insulation of the growing point. On the other hand, the trees are successfully cultivated across a wide range of soil types. They are able to trap water in rapidly draining soil by their deep network of roots. Date palm root development and distribution depends on soil characteristics, type of culture, depth of the underground water and genotype. For example, date palm roots are found as far as 25 m from the tree and deeper than 6 m, with 85% of the roots being distributed in the zone of 2 m deep and 2 m on both lateral sides in a light soil (Zaid and de Wet 2002d). These traits are examples of features that contribute to reduce evaporation rate and maximize water uptake, and are considered to play an important role in the adaptation of date palm to drought and high temperature. However, the tree requires a copious water supply for optimum yield and sustainable production (Nixon 1951, Martin 1992, Wickens 1998, Liebenberg and Zaid 2002, Ramoliya and Pandey, 2003). Research related to date palm's response to water availability remains concentrated on the effects on yield performance and productivity apart from the mechanisms concerning photosynthetic performance and growth capacity, especially under drought stress.

1.4. Aims of the research

Provided that date palm breeding is hampered by long regeneration cycles and complex cloning systems, the principal aim of the present study was to improve date palm production in Sudan through providing basic information on its genetic background, diversity and population structure, and to contribute to understanding the genetics of date palms in general. As date palm is a drought-tolerant tree, a further aim was to study the functional and structural behavior of this tree when subjected to water stress compared to well-watered conditions. The identity of date palm germplasm through a molecular marker system was investigated in **paper 1**. The genetic diversity and genetic structure of date palm germplasm and populations were studied in **papers 1 and 2**, where genetic structure was related to geographic locations and to the ecological features of date palm culture in Sudan. In **paper 3**, the apparent diversity of the most popular date palm cultivars in Sudan was further evaluated by employing different marker systems, including chemical characters of fruits and DNA polymorphisms to be utilized as descriptors of date palm cultivars, used either for individual cultivars or cultivar groups. The specific aims of **paper 4** were to evaluate the morphological and physiological adjustments of selected date palm cultivars to water stress, to identify the possible adaptive traits that enable the different ecological distribution of soft and dry types of cultivars, and to explore the mechanisms involved in the effects of water stress on photosynthetic gas exchange in date palms in general.

2. MATERIALS AND METHODS

2.1. Study area and date palm production

This research was conducted in Sudan as well as Morocco. In Sudan date palm culture is concentrated between latitudes 15.5-22^{o} N, especially along the River Nile banks. The study included also samples from isolated date palm sites in the oasis areas in northern Kordofan and in the eastern region of the country (Fig. 2). Date palm is cultivated mainly in the northern part of the country, where it is considered as the most important fruit tree, cultivated for more than 3000 years (Osman 2001) for its edible fruits. In addition to the commercial importance of the fruit, almost every part of the plant may be utilized in the rural economy for food, fuel, building purposes, animal feed, handicrafts and many other uses. In most cases, date palm trees are grown as the main constituent in a mixed agricultural system.

The long, hot and dry summers with low relative humidity and very low annual rainfall make northern Sudan an ideal location for date production (Osman 1984). Several cultivars have been recognised and selected by farmers, then cloned and cultivated for commercial production and local use. It has been reported that in Sudan there are about 400 varieties and strains of date palm, including dry, semi-dry and soft dates (Osman 1984). Moreover, there is a high potential for increasing the production area of date palm to cover local consumption in the whole country, and to produce date fruits for export purposes. The potential for date palm cultivation is getting more important with the increasing interest to expand its culture throughout the country south to the latitute 13^{o} N (Osman 2001).

Over the last 20 years, date palm culture in northern Sudan has been stressed by severe frequent River Nile floods and riverbank erosion, followed by subsequent spread of diseases, as well as by the increasing effect of desertification (United Nations Environment Programme 2007). However, date production in Sudan has increased from 120 000 tons during the years 1990-1994 (area harvested about 17 000 Ha) to 300 000-330 000 tons during the years 2000- 2007 (area harvested about 34 000 Ha; FAOSTAT

2009). Fig. 3 A. and B. show date palm trees on Elbaoga and Abu-Hamad sites, respectively, where date palm culture concentrates along or near the River Nile banks; Fig. C. shows date palm trees on the Gaab-Elsawani site, which is an example of date palm culture under very dry conditions and shows also the apparent effect of desertification; Fig. D. shows date palm culture in an oasis area in north Kordofan, on the Elbasheery site. Fig. 3 A, B and D show examples of date palm grown in mixed culture with citrus, vegetables and fodder.

The general features of date palm culture in Morocco are not much different from those in Sudan, especially when climate, cultivation and utilization concerned. It has been reported that in Morocco there are about 220 cultivars including different types of soft and dry dates (Zaid and de Wet 2002d, Bendiab et al. 1998). Drought, salinity, desert development and old age of palm trees are the principal constraints that restrict date palm culture in Morocco (Baaziz et al. 2000). However, the most known serious biotic stress reported in the history of date palm culture in North Africa was the Bayoud disease, a vascular fusariosis caused by *Fusarium oxysporum* f. sp. *albedinis*. Due to this disease 2/3 of the Moroccan date palm grooves were destroyed in one century (Baaziz et al. 2000), while very good cultivars disappeared. Date production in Morocco varies greatly due to variation in rainfall (Baaziz et al. 2000); it has decreased from 74 824 tons during the years 1990-1994 (average area harvested about 23 180 Ha) to 53 798 tons during the years 2000- 2007 (average area harvested about 33 538 Ha; FAOSTAT 2009).

2.2. Molecular markers and genetic characterization

2.2.1. Genetic diversity and identity of date palm cultivars (1)

To answer the question of how to confirm the genetic identity and true to typeness of each cultivar and to test their genetic diversity as well as their relation to the type of dates produced in each area, 37 cultivars collected from different locations in Sudan (Table 1, paper 1); eight cultivars collected from Morocco (Fig. 2), which include six from Zagora (30^o 33’ N, 5^o 86’ W) and two from Agdz in the same longitude with Zagora following Draa River (Baaziz et al. 2000). While 23 male samples collected from different locations

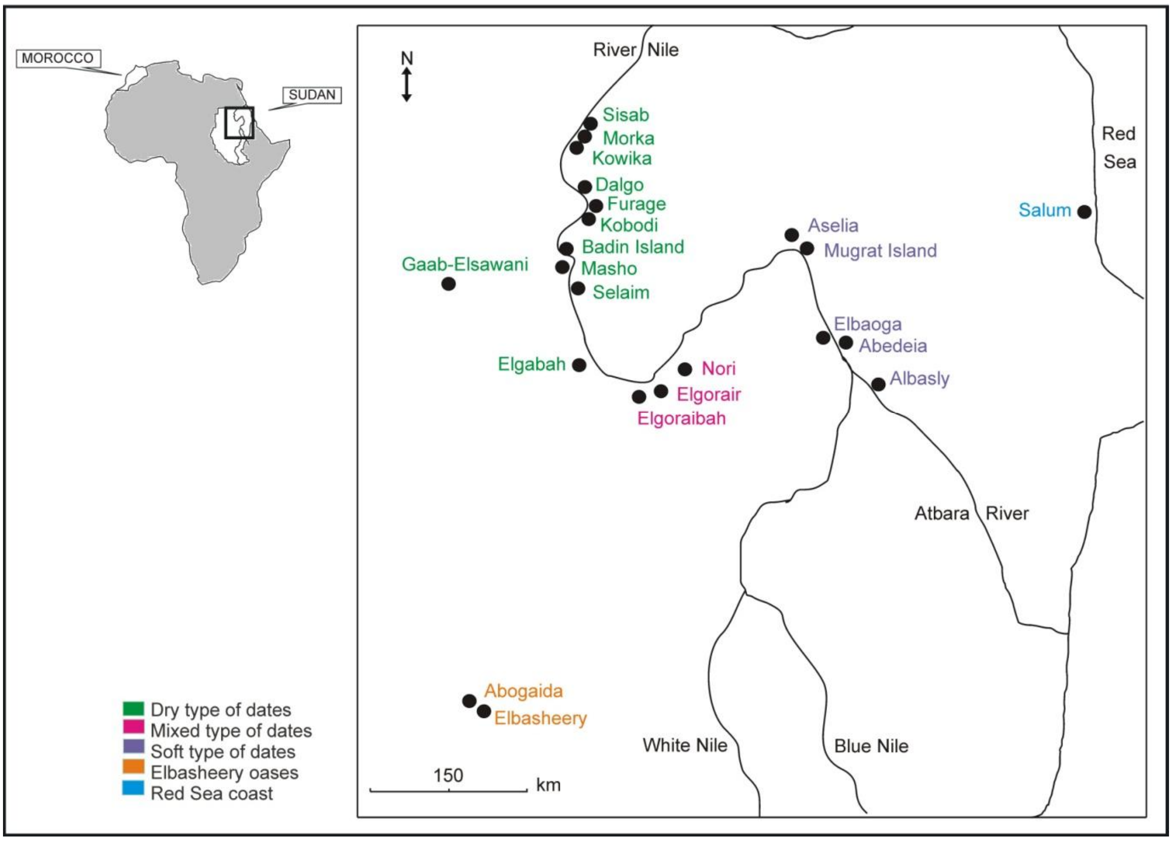

Figure 2. The sites of the collected date palm samples

Figure 3. A. Date palm trees in Elbaoga and B. Abo-Hamad sites, where date palm culture concentrates along or near the River Nile banks. Photo by Sakina Elshibli.

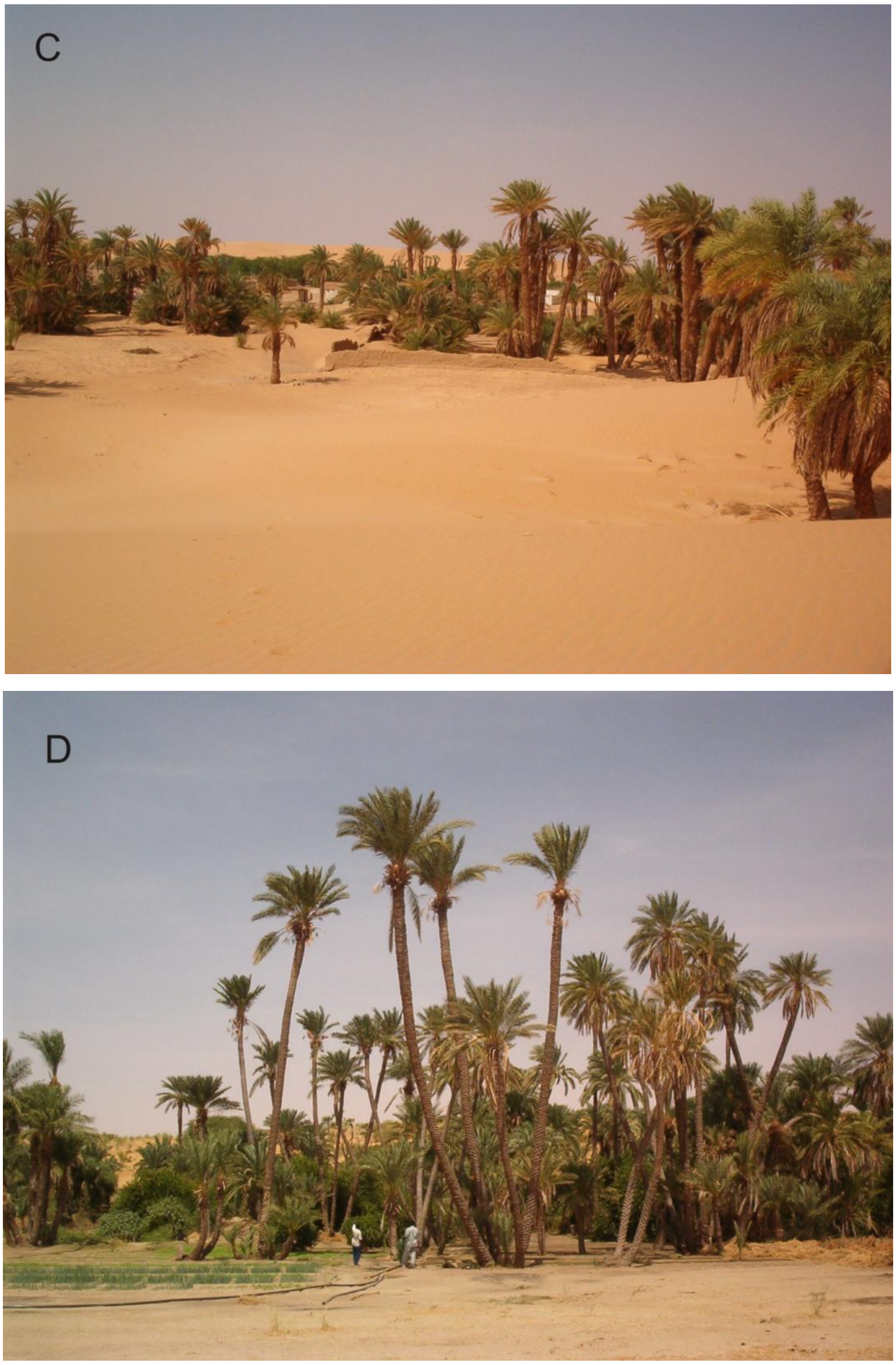

Figure 3. C. Date palm trees in Gaab-Elsawani site, which is an example of date palm culture under very dry conditions and shows also the apparent effect of desertification; D. Date palm culture in oases area in north Kordofan, Elbasheery site, an example of date palm mixed culture with vegetables, citrus and fodder. Photo by Sakina Elshibli.

in Sudan (Table 2, paper 1). Young leaves were collected from mature trees and dried and kept at room temperature until DNA extraction. Total genomic DNA was extracted from dry leaves, using DNAeasy Plant Mini Kit (Qiagen). DNA polymorphisms were detected by polymerase chain reaction (PCR) using 16 microsatellite markers developed for *Phoenix dactylifera* by Billotte et al. (2004). Ampilification products were genotyped using an automatic DNA analyser, MegaBace 1000 (Amersham Biosciences). Allele size scoring was performed by MegaBACE software, MegaBACE Genetic Profiler 2.2 (Amersham Biosciences 2003). Details of the PCR reactions and preparation of amplification products for genotyping are given in paper 1. The genetic diversity parameters were estimated. Based on the primary data, a distance matrix was calculated according to Nei's standard genetic distance (1972). Genetic distances of female cultivars and males were tested for the level of significance with a pairwise t-test. Samples were also subdivided into five geographical groups. For pairwise comparisons between these groups, F_{ST} values were calculated. Each group was tested for the Hardy-Weinberg equilibrium. Support for the inferred tree topology was evaluated using the bootstrap approach.

2.2.2. Genetic diversity and divergence of date palm populations (2)

The genetic diversity within and among populations, the correlation between the genetic and geographic distances, as well as the extent of genetic divergence as related to different geographic location and to type of date production areas were studied in 19 populations of date palm. We collected 200 individuals from different geographic locations in Sudan (Table 1, paper 2; Fig. 2). Seeds were germinated in an incubator for two months under temperature condition of 27 ^{o}C. Young, healthy leaves were harvested for direct DNA extraction. Total genomic DNA was extracted from fresh leaves, using a DNAeasy Plant Mini Kit (Qiagen). Ten pairs of primers were used to amplify $(GA)_n$ microsatellite loci using markers developed by Billotte et al. (2004) for *Phoenix dactylifera*. Amplification products were genotyped using an automatic DNA analyser, MegaBace 1000 (Amersham Biosciences). Allele size scoring was performed by MegaBACE software, MegaBACE Genetic Profiler 2.2 (Amersham Biosciences 2003). Details of the PCR reactions and preparation of ampilification products for genotyping are given in paper 2. Descriptive statistics of the within and between locations genetic diversity, the estimator F_{ST}, deviation from Hardy-Weinberg equilibrium, analysis of molecular variance and the isolation by distance were calculated from the microsatellite data.

2.3. Exploring chemical, morphological and DNA polymorphism for possible identifiers (3)

Date palm fruits of fifteen cultivars were collected at harvest (Tamr stage) from the Nori Horticultural Orchard in the Northern State of Sudan for morphological and chemical characterization. Morphological and DNA polymorphisms of the mother trees were also investigated. Data collected from five trees per cultivar, 60 fruits per tree grouped as four replications, each replicate consisting of five observations, and each observation being an average of three samples of fruits. Fruit weight, flesh weight, and fruit and seed sizes were measured using an analytical balance and a fernier scale. Tree morphology in terms of stem length and diameter, number and length of leaves, number and length of pinnae as well as number and length of spines at the midrib base were also measured. The chemical analyses of fruits included the determination of the percentage of glucose, fructose and sucrose and also the examination of dry matter and titratable acidity. Details of chemical analyses are given in paper 3. The leaves of the mother trees of the fifteen cultivars were genotyped using 16 microsatellite markers developed for *Phoenix dactylifera* by Billotte et al. (2004; details in paper 3).

Morphological and compositional data were collected and analysed as a completely randomised design with cultivars as treatments. The analysis of variance was conducted by SPSS 12.0.1 for Windows to test the significance of variation between cultivars for each character. When overall cultivar effects were significant, as indicated by F-tests, differences between individual cultivars were determined using Duncan' s multiple range test (Steel & Torrie, 1980). Multivariate analysis and statistical correlations were also performed among the compositional and morphological characters.

Principal components analyses (PCA) were performed on the variance matrices for each group of characters. The significance of similarities between individuals of dry and soft types of cultivars for each group of characters was tested by the Mann-Whitney U test. A discriminant function analysis was used for combined chemical and morphological characters to provide a set of weightings that allow dry and soft types of date palm cultivars to be distinguished. Tests for data exploration were performed using SPSS 15.0. Descriptive statistics for genetic diversity were calculated using Genetix 4.05 (Belkhir et al. 2000). To evaluate the genetic relationship between different cultivars, a principal component analysis was conducted using the SAS 9.1 software (SAS institute Inc.).

2.4. Photosynthetic responses to drought and adaptive traits (4)

2.4.1. Plant material and experimental design

Seeds of five date palm cultivars (half-sibs) were collected from two locations in the Northern State of Sudan in 2003. Initially 100 seeds per cultivar were randomly collected. The cultivars Gondaila, Barakawi and Bitamoda representing dry types were collected from the Nori Horticultural Orchard in Nori (18^o 32' 45'' N; 31^o 54' 15'' E;), and Mishrig Wad Laggai (Laggai) and Mishrig Wad Khateeb (Khateeb) representing soft types were collected from a farmer's orchard in Elbaoga (18^o 17' 45'' N; 33^o 54' 30'' E;). Twenty seeds per cultivar were grown for germination in pure sand soil in an incubator for two months under temperature condition of 27 ^oC. Seedlings were individually transferred to plastic pots (2 L) filled with sand and peat (White Sphagnum Peat, H 1-3 von post) in a 1:2 by volume ratio. Seedlings were grown in a greenhouse under 25±2 ^oC, relative humidity of 65%, and photoperiod maintained at 12/12 hours daily dark/light.

Prior to experimentation, uniform healthy one-year old plants of each cultivar were transferred to bigger plastic pots (7 L) in a mixture of fertilized peat (White Sphagnum Peat, H 1-3 von post) and sand (3:1 by volume). Before the start of the water stress treatments, the plants were watered twice per week to field capacity. Plants were equally fertilized at different intervals during the growth experiment with N-P-K-ratio of 8-4-14. The water stress phase of the experiment started early May 2006. A randomized complete block design with two factors consisting of five cultivars and four watering regimes (100%, 50%, 25% and 10% field capacity) was established. Each treatment was replicated five times.

2.4.2. Gas-exchange measurements

Gas exchange measurements were carried out using CIRAS2 portable photosynthesis measurement system (PP Systems, Hitchin, Hertfordshire, UK) during early October 2006. Leaf temperature was adjusted to 25 ^{o}C. Light intensity was adjusted above plant light saturation capacity at 1000µmol m^{-2} s^{-1}, provided by Tungsten Halogen light source. Four plants were measured from each treatment, and five observations were taken from each measurement. Net photosynthesis and other parameters were measured from the upper fully expanded leaf of each plant. Sampled leaves within each plant were always of the same age and similar position. The A:Ci measurements started at a CO_2 concentration close to ambient (350 part per million; ppm) and continued down to 50 ppm through four steps, increased back to 350 ppm, after which the leaf was allowed to acclimate for five minutes, and then up again to about 1500 ppm in four steps. At each step, gas exchange variables were recorded after achieving steady-state conditions (240 s and 180 s for stabilizing and recording, respectively).

The photosynthetic curves plotted against intercellular CO_2 concentration (A/Ci curves) were analysed to estimate the maximum rate of RUBP carboxylation (V_{max}) and the maximum rate of electron transport (J_{max}), which in turn drives RUBP regeneration. V_{max} and J_{max} were together estimated by applying the A-Ci data to the model proposed by (Farquhar et al. 1980), and subsequently modified (Von caemmerer and Farquhar 1981, Sharkey 1985, Harley and Sharkey 1991, Harley et al. 1992) using Photosynthesis Assistant software (Dundee Scientific, Dundee, Scotland, version 1.2, 2007), where the best fit was solved by minimizing the parameters to produce a minimum sum of squares for the difference between experimental and model data. In the calculation of these parameters according to the model, the following equation is used to express the relationship between assimilation rate and internal CO_2. This relies on the concept that it is a minimum of any of the three factors; amount and kinetic properties of Rubisco (Wc), the rate of RuBP regeneration (Wj) and availability of inorganic phosphate (Wp) which limits CO_2 assimilation. That is:

$$A = \left(1 - \frac{0.5\,O}{\tau\, C_i}\right) \times \min(\mathrm{Wc}, \mathrm{Wj}, \mathrm{Wp}) \ - \ \mathrm{R}_{\mathrm{day}}$$

where R_{day} refers to the release of CO_2 in the light by processes other than photorespiration and may be estimated using the modelling equations below:

1. The rate of carboxylation limited by the amount and kinetic properties of Rubisco is given by:

$$Wc = \frac{Vc_{\max}.Ci}{\left[Ci + Kc(1 + O/Ko)\right]}$$

where Kc and Ko respectively are the Michaelis-Menten constants of Rubisco for CO_2 and O_2 and O is the oxygen partial pressure in the intercellular spaces.

2. The rate of carboxylation limited by the regeneration of RuBP as a function of the rate of electron transport, and is given by:

$$W_j = \frac{J.C_i}{4(C_i + O/\tau)}$$

Tau (t) represents the specificity factor of Rubisco for CO2/O2 The factor 4 represents the fact that four electrons will generate the three ATP (adenosine triphosphate) and two NADPH (nicotine adenine dinucleotide phosphate that acquires a hydrogen ion) required in the Calvin cycle to regenerate RuBP. While, J is the potential rate of electron transport through PS11 (Photosystem 11) given by:

$$J = \alpha.I \div \sqrt{1 + (\frac{\alpha.I}{J_{\max}})^2}$$

Where α is the efficiency of light conversion and J_{max} is the light saturated rate of electron transport and I is the incident radiation.

3. The rate of carboxylation limited by the regeneration of inorganic P and can be described by:

$$W_p = 3(TPU) + \frac{0.5 \times Vo \times O}{C_i \times \tau}$$

Where Vo represents the rate of oxygenation of Rubisco.

Analysis of variance (ANOVA) was used to analyse the morphological adjustments that take place in response to water treatments, and as affected by cultivar, phenotype (soft and dry types of cultivars), as well as their interactions. ANOVA was also used to analyse the effects of the tested cultivars, types of cultivar irrigation water and external supply of C_a on photosynthesis and photosynthetic parameters stomatal conductance (gs) and Ci. A stomatal limitation index (Sage 1994, Drake et al. 1997) was estimated by calculating the Ci/ C_a ratios across all water treatments. Two-way ANOVA was performed to test the interaction effect of water and C_a on photosynthesis. Comparisons between means were conducted by Duncan test, except for the external supply of C_a, where Bonferroni adjustments method for multiple comparisons was used (Chan 2003). ANOVA was also used to test the differences in the photosynthetic biochemical parameters (V_{max} and J_{max}) of date palm plants in response to irrigation water, where three replications were considered. Pearson's correlation was also performed among some variables. Principal component analyses (PCA) were conducted on the variance matrices for different responses to irrigation water, including photosynthetic as well as morphological characters. The significance of similarities between individuals of dry and soft types of cultivars for each group of characters was tested by the Mann-Whitney U test. All tests for descriptive statistics and data exploration were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1 Molecular markers and genetic characterization

3.1.1. Genetic diversity and identity of date palm cultivars (1)

The genetic characteristics of the Sudanese date palm cultivars were also compared to selected cultivars from Morocco. The microsatellites examined were highly polymorphic possessing a great number of alleles with an average of 21.4 alleles per locus (Table 3, paper 1). The numbers of alleles per locus detected in this study were higher than those scored by Zehdi et al. (2004a) for 46 Tunisian date palm accessions, for which only 100 different alleles were identified at 14 microsatellite loci. The mean expected and observed heterozygosity detected for the Tunisian date palms were 0.70 and 0.61, respectively, while the respective values detected in this study equaled 0.853 and 0.912. Our results indicated the presence of higher genetic diversity in the Sudan date palms compared to the Tunisian date palms, which may be explained by intensive selection operations in some of the Tunisian date palm oases (Zehdi et al. 2004a). The genetic diversity of Sudan date palms is strongly represented within groups rather than between groups. This result agrees with other reports for the Moroccan, Algerian and Tunisian date palm cultivars based on analyses using microsatellite markers (Zehdi et al. 2004a) and isozyme markers (Bennaceur et al. 1991, Salem et al. 2001).

Sudan date palm groups as well as Morocco group possessed somewhat more heterozygous individuals than expected (Table 5, paper 1), which may be due to extensive distribution of date palm offshoots to new areas at times. However, the deviations of the observed heterozygosity from the Hardy-Weinberg equilibrium were generally non-significant. The microsatellite loci exhibited low F_{ST} values among different groups of Sudan date palms (Table 6, paper 1), which shows that the observed high genetic variability is maintained locally within groups. On the other hand, at a big number of alleles, as observed in this study, the probability of identity of two different loci decreases, which results in a reduced F_{ST} values (Hedrick 1999, Slatkin 1995]. However, these values agreed with those observed by Zehdi et al. (2004a) in four groups of date palm collections from Tunisia. The significant differentiation of the Morocco cultivars in relation to the Sudan groups, as measured by F_{ST} values and genetic distances, is indicative of the geographic effect on differentiation, although the cluster analysis showed weak clustering relationships and no specific grouping. Comparable relationships have also been reported by Sedra et al. (1998), Cao and Chao (2002) and Zehdi et al. (2004b), who independently tested samples from Tunis, California and Morocco with inter-simple sequence repeat (ISSR), AFLP and RAPD markers.

The long history of date palm domestication with an unknown origin (Wrigley 1995) and the nature of date palm culture may have played an important role in the composition of date palm genomes. Some authors have suggested a common genetic basis among date palm genotypes in spite of their distinction related to fruit characters and tree morphology (Sedra et al. 1998, Trifi et al. 2000, Zehdi et al. 2004a). Moreover, environmental effects, and interactions between environmental and genetic factors may strongly contribute to the fruit traits of date palm. Specifically in Sudan, there is no concrete, organised cultivar selection. The method of pollination is mainly mechanical. Farmers depend on few selected males for the pollination of female trees. Male trees are selected for fruit quality and they are exchanged between farmers (Osman 2001). Pollen grains of more than one male are sometimes mixed and used then for pollination. New cultivars are a result of a continuous selection process carried out by farmers in their fields following sexual reproduction. Exchange of propagules, which are a mix of vegetative and seed-propagated materials, is conducted between farmers. In Morocco when Bayoud disease destroyed the best date cultivars farmers allowed date palm seeds to grow in places where other seasonal crops were growing. It is found difficult to distinguish trees from seedlings with the traditional cultivars, since fruits of similar quality were obtained from both materials (Bendiab et al. 1998). All these processes together may result in a mixed genome of date palm within each country.

3.1.2. Genetic diversity and divergence of date palm populations (2)

The presence of high genetic diversity among date palm cultivars has been reported in Tunisia and Sudan (Zehdi et al. 2004a, Elshibli and Korpelainen, 2008). However, these studies were based on individual collections to investigate genetic relationships between cultivars; all studied samples consisted of leaf material used as references to cultivars (females). The high intrapopulation genetic diversities (mean 0.809) detected in this study indicate that the studied populations possess an abundant source of variation that is needed for evolutionary responses in a changing environment and for breeding purposes to improve the agronomical and commercial characters of date palm. The observed levels of genetic diversity are in line with the levels reported in other woody, wind pollinated tree species. Zhang et al. (2006) reported an average expected heterozygosity equalling 0.779 among five natural populations of *Quercus aquifolioides* examined with microsatellite markers. Moreover, tree species with both sexual and asexual modes of reproduction may maintain higher levels of genetic diversity (Hamrick et al. 1992), as it was observed in this study on date palm with the mean H_{exp} equalling 0.851.

The 19 tested populations possessed more heterozygous individuals than expected, as indicated by the negative mean values of fixation indices. This may indicate a continuous process of small populations becoming mixed together or the presence of other evolutionary processes, such as selection. This result is in line with our previous study on the genetic diversity of a wide range of date palm cultivars (Elshibli and Korpelainen 2008), in which we suggested that the exchange of off-shoots (propagules) of date palm cultivars may result in heterozygous populations. However, the distribution of genetic constituents may result also from the introduction of seedlings after fruit consumption. Yet, the deviations of the observed heterozygosity from the Hardy-Weinberg equilibrium were generally non-significant, indicating that the evolutionary forces proposed by Hardy-Weinberg theory does not affect the genetic equilibrium of date palm in Sudan in spite of the mixed cloning and sexual reproduction systems, male selections for pollination as well as the exchange process of dispersal agents.

In general, the microsatellite loci exhibited low F_{ST} values among different populations of date palms, which show that the observed high genetic variability is maintained within populations. Also F_{ST} values may reduced due to the effect of high number of alleles per locus. These values agree with those observed by Zehdi et al. (2004a) and Elshibli and Korpelainen (2008) in different groups of date palm cultivars collected from Tunisia and

Sudan. According to the measured F_{ST} values, there is no clear trend in the differentiation of populations originating from different locations (Table 3, paper 2). For example, the distinct differentiation of the Salum population (mean F_{ST} = 0.116; p<0.001) from all other 18 populations is not surprising and can be mainly attributed to the effect of geographic distance (2530-2015 km). Other characteristic features of the Salum location on the Red Sea coast include the rainy winter season and the altitude of the Red Sea Hills (up to 2 000 m; Google Earth 2008, Babiker and Gudmundsson 2004) situated between the Salum location (173 m) and the River Nile banks.

Pollination of date palm naturally takes place by wind, bees and insects and is found to yield a fair fruit set in various areas within the date palm growing countries (Zaid and de Wet 2002a). Natural pollination of date palms is characterized by 100% seedling composition. However, commercial date production necessitates artificial pollination. Date growers are aware of the importance of artificial pollination and practice it according to different traditional methods or by using a mechanized device. Pollen grains selected from male trees according to the well verified effects of Xenia (effect of pollen grains on the size of fruits and seeds) and Metaxenia (effect of pollen grains on the time of fruit ripening; Ahmed and Ali 1960, Nixon and Carpenter 1978, Zaid and de Wet 2002a). On the other hand, pollen grains are usable as fresh or dried and can be stored for the next season as well (Zaid and de Wet 2002a). In this regard, the dispersal of pollen grains is expected to be more extensive, and the exchange process between farmers is expected to be more effective along the River Nile rather than in the Salum location, where the dispersal of pollen grains may remain effective only within the location. Some reports have shown a positive correlation between the presence of specific, low-frequency alleles and elevation in some plant species (Zhang et al. 2006). However, in this study, the differences in elevation in the other locations than around Salum were minor (range 217-500 m above sea level). On the other hand, there is no specific knowledge of the effect of elevation-related factors on date palm.

The significant differentiation between the two populations from the Elbasheery oases, accompanied by nonsignificant differentiation of one of these populations from date palms in the Sai Island (Sisab population; Fig 1) was surprising, especially if we consider the distance between the two sites (750 km) and the location of the Elbasheery oases apart from the River Nile and other date palm production areas. It is clear that some factors other than geographic distances play an important role in affecting the gene flow and genetic structure of date palm populations world-wide. These factors may include the exchange of propagation and/or pollination material, and seed dispersal. The populations in the Elbasheery oases showed moderate gene flow (F_{ST} = 0.05) compared to other groups of populations. The isolation by distance from other populations present along the River Nile may cause difficulties in exchanging and dispersing plant materials to those locations. This situation may lead to the development of small isolated populations of date palms in the Elbasheery oases and highlights the need for better management of date palm culture in that region.

The observed high degree of genetic variability within populations compared to relatively low levels of genetic differentiation among the nineteen populations, detected also when the groups were analysed separately, may confirm the idea of effective dispersal, which decreases population structuring. In date palm, the dispersal agents include seed, pollen grains and off-shoots. Offshoots and pollen grains are mainly distributed between farmers within a village, province or country, while seed dispersal takes place by travellers and traders across geographic boarders. The dispersal effect on the effective population size is a function of the variance in dispersal distances (Loveless and Hamrick 1984). Long-distance seed dispersal and propagule establishment prevent population divergence. On the other hand, date palm trees have a characteristic long-lived cycle in nature. Although the average economic life of a date palm tree is estimated to be up to 50 years, the tree may stay productive up to 150 years (Chao and Krueger 2007). Such long generation times also increase the effective population size and prevent population divergence (Loveless and Hamrick 1984).

The presence of considerable variability between date palm populations as related to the type of date production area agrees with the general classification of these areas according to the morphological and chemical characters of fruits and cultivars (Fig. 4)

When moving from northern Sudan at the Egyptian border to the south, the type of date palm fruits tends to change from dry type to semi-soft and to the soft type (Fig. 2). This trend seems to follow the degree of aridity, varying from the arid region in the north to the semi-arid region in the central part of the country (Hulme 1990), including the Khartoum area where all commercial date production comprises soft type dates. Unfortunately, the Khartoum area was not included in this study because of a failure in seed germination. Although our results significantly contribute to the knowledge of date palm, it is clear that the classification of different types of dates related to production area and the characteristic environmental features need to be investigated further in detailed studies in the future.

Significant ($p < 0.05$) isolation by distance, revealed by a Mantel test, was observed. This observation was detectable for the Salum population as indicated by the significant pairwise F_{ST} values compared to all other populations and the large geographic distance that separate Salum population from others (maximum and minimum distances to other locations are 2530 and 2015 km respectively). However, it seems that the spatial effect has become complicated as a result from the exchange and introduction of different kinds of plant material by date palm growers and traders. This complexity was apparent in the weak clustering relationships among most of the tested populations, while a close relationship was detected, e.g., between the populations from the Badin Island and Salum on the Red Sea coast. Comparable relationships have also been reported by Sedra et al. (1998), Cao and Chao (2002), Zehdi et al. (2004b) and Elshibli and Korpelainen (2008) who independantly tested cultivar samples from Tunis, California, Morocco and Sudan with different molecular markers.

3.2. Exploring chemical, morphological and DNA polymorphism for possible identifiers (3)

3.2.1. Morphological and compositional characters

A large amount of diversity was found among the tested 15 date palm cultivars at morphological, chemical and genetic levels, including tree and fruit characters ($p < 0.001$). Although this variability provides a wide range of choices for selection and adaptation of date palm cultivars, it makes the screening and documentation of all existing cultivars very difficult. Date palm culture in the world has been based on the development of thousands of cultivars exhibiting a wide range of variability in terms of fruit characters and tree morphology. The distribution and strength of this variability vary from one country to another resulting in different criteria and names of thousands of date palm cultivars (Jaradat and Zaid 2004). The popular and marketable characters of some cultivars have encouraged date palm growers for extensive exchange habits of date palm cultivars within each producing country and overseas as well (Osman 2001; Jaradat and Zaid, 2004). Within Sudan, date palm culture is based on the use of varieties and strains which are estimated to number 400 (Osman 1984).

Some cultivars are distinguishable by their growth habits which include both the pattern and vigour of growth (Elhoumaizi 2002). These characters constitute an important part of characterization in Tunisia (Bioversity International 2008). In Sudan, for example, a well known single character that differentiates the growth of the Laggai and Khateeb cultivars is the orientation of spines along the base of the leaf (either alternate or opposite arrangement, with two spines or a single spine, respectively), but this character is not stable in other cultivars. In this study, the Laggai and Khateeb cultivars belonged to significantly different groups (Duncan test; $p < 0.05$) regarding to stem diameter, tree height, and the number of leaves and spines. However, data exploration through PCA indicated specific trends in different types of dates related to tree morphology ($p < 0.001$), which confirms the importance of multivariate analysis when high diversity exists.

Regarding to fruit morphology (Fig. 5A1), there was an agreement with farmers' and consumers' characterization. For example, fruit size was found to be one of the characteristic features that differentiate cultivars into distinct groups. It is familiar among farmers and local consumers that Gondaila and Bitamoda possess big fruits and are rich in fruit flesh. However, the same cultivars may significantly vary in their fruit size in different locations and seasons as a response to environment and to cultural practices (Bashab 1997, Baballa 2002).

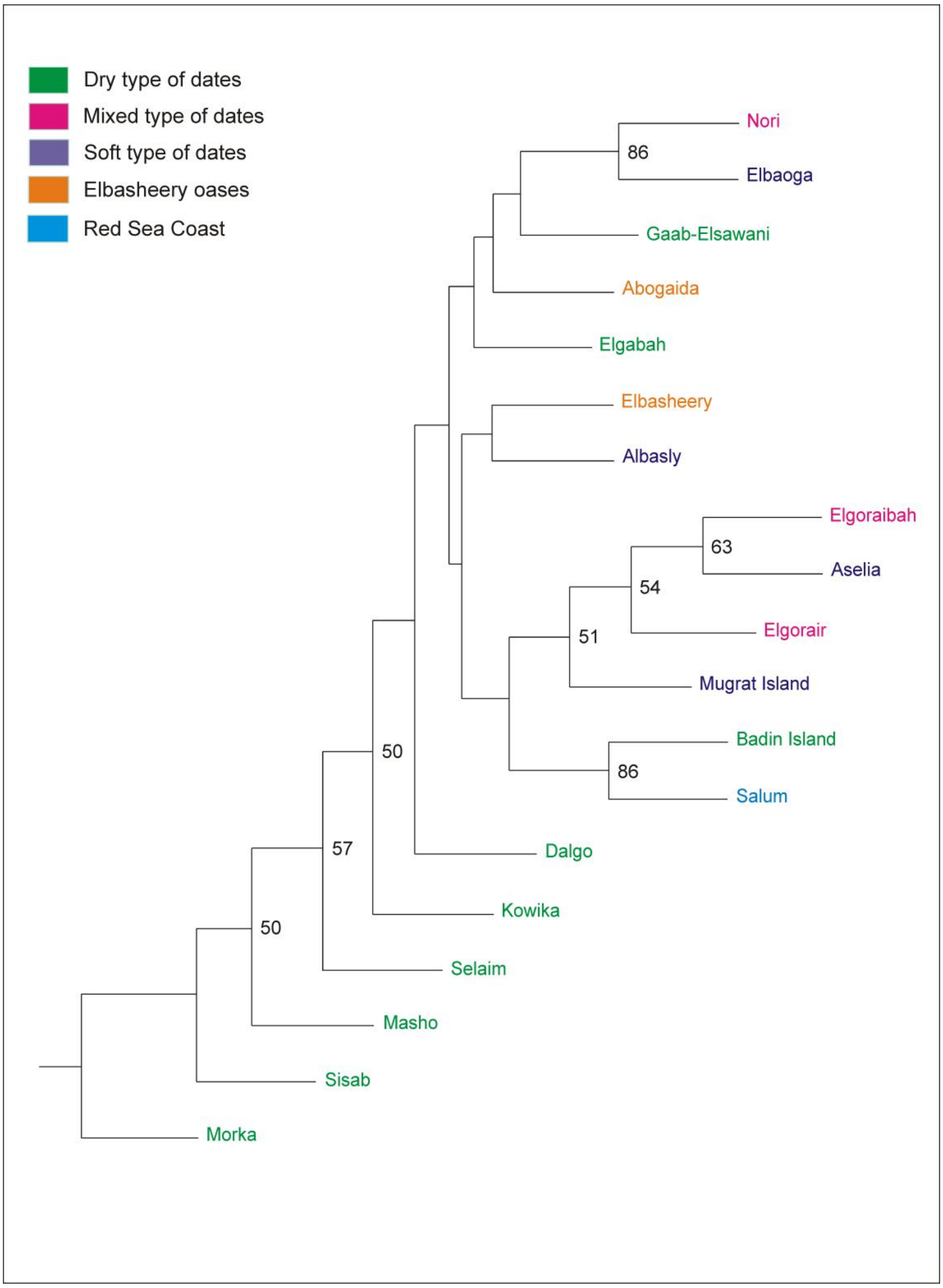

Figure 4. Neighbour-joining tree illustrating the relationships among 19 populations of date palms collected in Sudan. The tree is based on Nei's (1972) pairwise genetic distances. The primary allele frequency data were bootstrapped 1000 times. Only bootstrap values of at least 50% are shown. Variation among groups is significant at $p < 0.01$ (AMOVA).

The Medina, Laggai and Khateeb cultivars are classified as belonging to the soft type of dates in Sudan (Osman1984). Consistent with this classification, these three cultivars contained the lowest percentage of dry matter. Tonisi, followed by Asada, Gondaila and Shidda, possessed the highest dry matter percentages. These cultivars are known as the dry type of dates (Bashab 1997, Osman 1984), despite the lack of practically any previous data on their dry matter and sugar contents. In this study, soft types of dates were clearly separated from dry types when chemical characters were considered in PCA with main contribution of sucrose and dry matter (48% on PC1; Fig 5A2).

Negative loadings of glucose and fructose were observed on PC1 which can be explained also by the negative correlation ($p < 0.01$) of dry matter concentration to glucose and fructose concentrations, and the positive correlation ($p < 0.01$) of sucrose and dry matter content (Table 3 Paper 3). This correlation of dry matter versus sucrose content may explain the classification of dates into soft and dry types, which, thus, seems to be related to the sugar and dry matter contents at the final stage of ripening. The increase in reducing sugars and the decrease in sucrose from stage 1 to stage 4 in relation to the decrease of the water content in dates during these stages (Al-Shihab and Marshall 2003) may depend on the cultivar in question, especially in the dry types. This suggests that these characters are highly affected by the environment, stage of ripening and the cultivar itself, or by the interaction of these factors. On the other hand, the negative correlation of sucrose and reducing sugars also follows the hypothesis of sucrose conversion into glucose and fructose due to invertase activity during fruit ripening towards the Tamr stage (Hulme 1970).

Despite the well known fact that farmers select date palm cultivars according to their appearance, taste and flavour, there are no reports available on the acidity and/or the type of acids that dominate in the fruits of different cultivars of dates. In this study, acidity was found to be a distinguishable character for almost every individual cultivar. Among the 15 cultivars, 13 significantly (Duncan's test; $p < 0.05$) different groups were observed (Fig. 1; paper 3). Further studies are needed to determine to what extent acidity is an important in variety assessment for commercial purposes, provided that unexplained large variation exist in the taste of date palm fruits worldwide (Zaid and de Wet 2002d).

3.2.2. Microsatellite polymorphism

The high level of DNA polymorphism, as indicated by the high expected (0.762-0.920) and observed (0.733-1.000) heterozygosity, seems to be a general characteristic feature of date palm germplasm (Zehdi et al. 2004a and b, Elshibli and Korpelainen 2008, 2009a), in almost all producing countries. However, the high genetic variability is not associated with specific genetic relationship in most of the tested cultivars (Sedra et al. 1998, Cao and Chao 2002, Zehdi et al. 2004b, Elshibli and Korpelainen 2008), the maximum genetic distance was observed between the Meddina and Bitamoda cultivars (3.496), while the minimum genetic distance was observed between the Laggai and the Asada cultivars (0.693). Each of these relationships constitute a relationship between cultivars that belong to soft and dry type of dates.

3.2.3. Overall effects and ecological distribution

The results of the principal component analysis confirmed that the grouping of Sudan date palm cultivars is mainly based on the chemical and morphological characters of fruits and tree morphology. Using the fifteen cultivars and all chemical and morphological characters of the fruits, the discriminant analysis correctly predicted the dry type of date palm cultivars (100%) with probabilities of membership ranging between 54% for the Bitamoda cultivar to 99.9 % for all other cultivars, while the probabilities of membership within soft type of dates were 100% for all cultivars. According to the standardized coefficients, the highest weightings that maximize the differences between groups were observed for chemical characters (8.93 - 3.89) and seed weight (-6.97). The distribution of date palm culture in Sudan follows a geographic pattern including locations for the successful production of either soft or dry type of dates (Osman 1984). The combined effects of different characters of each marker system on the scatter plot of tested cultivars based on the two first principal components were compared (Fig. 3 Paper 3). A distinguishable grouping pattern of cultivars that belong to soft type of dates and those belonging to dry type of dates was observed for chemical characters of fruits (Z =

2.064, p = 0.039; Fig. 3a), fruit and seed morphology (Z = -10.204, p = 0.000; Fig. 3b) and tree morphology (Z = -3.778, p = 0.000; Fig. 3c). This relationship was detectable when we studied the population genetics of date palms in Sudan (Elshibli and Korpelainen 2009a), where population seed samples were collected from both types of locations. However, among the 15 tested cultivars a comparable relationship was not observed for DNA polymorphism (Z = -0.392, p = 0.695; Fig. 3d). It is worth to mention that in this study, our leaf samples were individual cultivars, representing the mother trees, collected from Nori Horticutural Orchard, a location considered suitable for the production of both types of dates.

3.3. Photosynthetic responses to drought and adaptive traits (4)

3.3.1 Growth and morphological traits

Reducing irrigation water in date palm plants resulted in multiple changes in plant growth and morphology, the changes being most pronounced at 10% and/or 25% of field capacity (Table 1, paper 4). The observed increase in the number of dry leaves indicated one of the drought adaptive mechanisms, accelerated leaf senescence, which contributes to plant survival under drought stress and allows remobilization of nutrients from senescing leaves to young leaves, and reduction in water loss at the whole plant level (Munne-Bosch and Alergre 2004). Soft types tend to maximize the water uptake from soil by a slight increase in the root/shoot ratio at 50% FC resulting from a significant increase in root dry weight and a slight decrease in shoot dry weight (Table 1, paper 4), however, at higher levels of water stress both phenotypes showed decrease in root/shoot ratios. An increase in root to shoot ratio, attributable mainly to a reduction in shoot growth, has often been observed when water is limiting as resource allocation to roots on the expense of shoots (Sharp and Davies 1979, Pereira and pallardy 1989, Susiluoto and Berninger 2007). The growth rate of wheat and maize roots was also found to be decreased under moderate and high water stress (Shao et al 2009). In line with these results, Djibril et al. (2005) reported that root system may increase the water uptake and maintain the correct osmotic pressure through higher proline levels. The decrease in total biomass (Table 1 paper 4) under stress of 10% FC resulted from the direct slowing down of the whole growth and optimization of nutrient utilization (Osorio et al. 1998), indicated by the decreased number of green leaves, induced by early senescence of older leaves (Munne-Bosch and Alergre 2004), and the reduction in the number of photosynthesizing pinnae.

The response of the tested cultivars and phenotypes to water availability and their interactions showed different patterns, however, number of pinnae, plant height and root dry weight were the traits that have cultivar and/or phenotype effect other than drought effects (Table 1, paper 4). These traits may be considered to play a role in adaptation of each phenotype, which may represent a specific genotype, and can be considered for selection processes when tolerance to drought addressed. Cultivar-specific morphological responses to water deficit induced by polyethylene glycol was reported in date palm, at early stages of seedlings development (Djibril et al. 2005) as well as in *in vitro* callus cultures (Al-Khayri 2004).

3.3.2. Physiological traits

For ambient C_a (350), the C_i/C_a ratio for the studied date palm plants, across all water treatments, was lower than the expected values of C_i/C_a ratio for C_3 plant species (Fig. 2C paper 4; Wong et al. 1979, Drake et al. 1998), and rather in the range of values expected for C_4 plants (Jones 1983, Lambers et al. 2008). As there is no previous data on the gas exchange and photosynthetic mechanism of date palms in general, this result needs to be validated, especially under natural conditions of date palm culture. The values of Ci/C_a ratios of some plant species have been found to vary greatly between different growth conditions, for eaxample, field versus greenhouse (Bunce 2005). However, date palms may possess a specific strategy for gas exchange and photosynthetic metabolism, perhas as it is a monocotyledonous desert tree. Measurements conducted under greenhouse conditions, at 350 ppm for 25 different, well-watered cultivars originating from other areas of collection in Sudan possessed Ci/Ca ratios also in the range of 0.3 - 0.4 (S. Elshibli, nonpublished data), which confirms the present results.

The reduction of gs by 30% at 10% FC compared to 100% did not affect the increasing pattern of Ci/ C_a ratio of elevated CO_2 when compared to that of 350 ppm. Comparable Ci/ C_a responses have been previously reported in water-stressed plants. Apart from gs effect, the increase in Ci/ C_a may result from CO_2 that evolves from leaves due to maintenance of light respiration (Haupt-Herting and Fock 2002, Lawlor and Tezara 2009), as a result of inhibition of photosynthetic metabolism affected directly by water stress (Farquhar et al. 1982, Lawlor and Tezara 2009). Although photosynthesis was lower at 25% and 10% FC when compared to other treatments, the continuous increase in Ci (Fig. 2B and Fig. 3 paper 4) was accompanied by a comparable increase in photosynthesis (Fig. 3 paper 4), which confirms the non-stomatal inhibition of photosynthesis (Drake et al. 1997, Tezara et al. 1999, Haupt-Herting and Fock 2002). On the other hand, the continuous increase in photosynthesis with Ci may also include partial reassimilation of the evolved photorespiratory CO_2, which is considered to maintain the carbon flux and the enzymatic substrate turnover, which enables the plants to recover rabidly after rewatering (Haupt-Herting and Fock 2002).

Metabolic impairement at water stress was apparent in the reduced V_{max} and J_{max} (Fig. 6A and B, respectively; paper 4). The changes in V_{max} and J_{max} resulted in an increasing J_{max} / V_{max} ratio, coupled with a shift in the $Ci_{inflection}$ of A/Ci curve to higher CO_2 concentrations (Fig. 5 paper 4). This situation indicated that as water stress increases, the photosynthetic rate of date palm plants tends to be more dependent on CO_2 concentration (Bernacchi and Morgan 2005, Onoda et al. 2005). Therefore, a greater stimulation of photosynthesis was observed with increasing CO_2 supply function, with water interaction effect. Manter and Kerrigan (2004) have observed in some woody plant species that when light is saturating, the actual rates of CO_2 assimilation are limited by the amount, activity and kinetics of Rubisco more often than electron transport rates, a situation detected when $Ci < Ci\_{inflection}$. The differences observed in A/Ci responses of date palm plants at drought stress of 10% FC compared to non-stressed plants suggest a strategic shift from RUBP regeneration limitation (100% and 50% FC) to RUBP carboxylation limitation, while, as suggested by Manter and Kerrigan (2004), some factor other than RUBP regeneration plays a role at limiting Rubisco activation. The changes in the

J_{max}/V_{max} ratio observed in this study were within the range of values reported by Wullschleger (1993), V_{max} from 6 to 194 µmol m^{-2} s^{-1}, and J_{max} from17 to 372 µmol m^{-2} s^{-1} across109 plant species with a strong correlation between these two parameters. According to Onoda et al. (2005), this relationship can be linearly presented across a range of woody plant species with r^2 =0.827, compared to our results with r^2 =0.884 across different water treatments, indicating that CO_2 assimilation is regulated in a co-ordinated manner by these two processes (Wullschleger 1993).

The adjustment of biochemical reactions apparent in the co-ordinated reductions of V_{max} and J_{max}, the possible reassimilation of evolved photorespiratory CO_2 (Haupt-Herting and Fock, 2002) and utilization of triose-phosphate capacities apparent in the observed increase of photosynthesis with increasing Ci, can be considered as a long-term adaptation of the photosynthetic machinery to water stress in date palm plants, allowing drought-stressed plants to escape photodamage by downregulating photosynthesis (Pankovic et al., 1999; Maroco et al., 2002).

3.3.3. Phenotype-specific responses

Dry and soft phenotypes showed significant differentiation in morphological and physiological traits in response to different levels of water availability (paper 4). It was apparent that soft phenotype has comparable longer plants and less number of pinnae (Table 1, paper 4), but with higher stomatal conductance even under well watered conditions (Fig. 4 B). Under water stress especially at 10% FC, the differentiation in plant height and number of pinnae among the two phenotypes decreased, while differentiation due to other growth traits mainly root fresh weight, root/shoot ratio and biomass traits increased (Table 1, paper 4). The more pronounced physiological adjustments that lead to differentiation of soft and dry phenotypes included the ability of soft types to fix more CO_2 in the supply function (Fig. 5 A3 and B3, paper 4) confirmed by the differences in the J_{max} / V_{max} ratio (Fig. 6 C, paper 4) and resulted in a significant differences in photosynthesis (Fig. 4A, paper 4).

The adjustments in different morphological traits were difficult to follow on individual bases. We tried to solve this observed complex pattern of morphological responses in each phenotype of date palm cultivars by applying the Principal Component Analysis. Apparent phenotype-specific responses were observed even at high levels of water supply ($p < 0.05$; Fig. 7A paper 4). Comparable results have been observed also in our previous study (Elshibli and Korpelainen 2009b), when we studied the biodiversity of selected date palm cultivars, including morphological and growth characters of other mother trees, and morphological and chemical characters of fruits as well. However, differentiation between these two phenotypes became more distinct under increasing stress conditions ($p < 0.001$ at 25% and 10% FC; Fig. 7C and D, paper 4), mainly due to different responses in numbers of pinnae (PC1) affected by increasing numbers of senescent leaves and the response of root/shoot ratio (PC2).

This result may indicate that phenotype-specific adaptation and survival strategies depend on the degree of stress and on the specific trait, and suggest that the responses of soft and dry types are enhanced by different genetic constituents that lead to different responses to drought stress. The influence of genotype on shoot morphology and growth has also been observed in some other plant species when subjected to environmental stresses (Munne-Bosch and Alergre 2004, Meier and Leuschner 2008, Izanloo et al. 2008). Fig. 5 shows comparable dry and soft types differentiation due to A: morphological traits under normal field irrigation conditions and B: differentiation due to physiological traits induced by water stress under greenhouse conditions.

On the other hand, two factors were found to explain 92% (57.9%; 33.8%) of the diversity of tested physiological traits, gs and Ci in response to different water treatments as well as C_a. Photosynthesis (0.94) and Ci (0.92) constituted the highest load on PC1, which explained 50% and 49% of the total variance, respectively, and gs (0.99) constituted the highest load on PC2, which explained 97% of the total variance. The combined effects of the three characters of each water treatment on the scatter plot of tested cultivars based on the first two principal components were compared. A distinguishable pattern of cultivars belonging to the soft type and dry type of dates was observed for water treatments of 25% and 10% FC ($p < 0.001$), while no specific pattern was observed for water treatments of 100% and 50% FC.

The differentiation among soft and dry types in photosynthetic capacity under severe water stress was observed also in A/Ci response curves (Fig. 6 A3 and B3), where dry types were found to be more sensitive than soft types. In line with these results, Xu et al. (2008) observed in *Populus cathayana* that drought inhibits photosynthetic capacity more in female genotypes than in male genotypes, and genotype-specific morphological and/or physiological responses to water stress have been observed also in bread wheat and apple cultivars (Massonnet et al. 2007, Izanloo et al. 2008). Previously, genetic differentiation ($p < 0.01$) between populations of soft and dry types of dates has been detected using microsatellite markers (Elshibli and Korpelainen 2009a). Genetic differentiation may cover specific traits that contribute to adaptive mechanisms in each phenotype. Further investigations to identify specific traits for drought adaptation as well as possible drought responsive genes are needed.

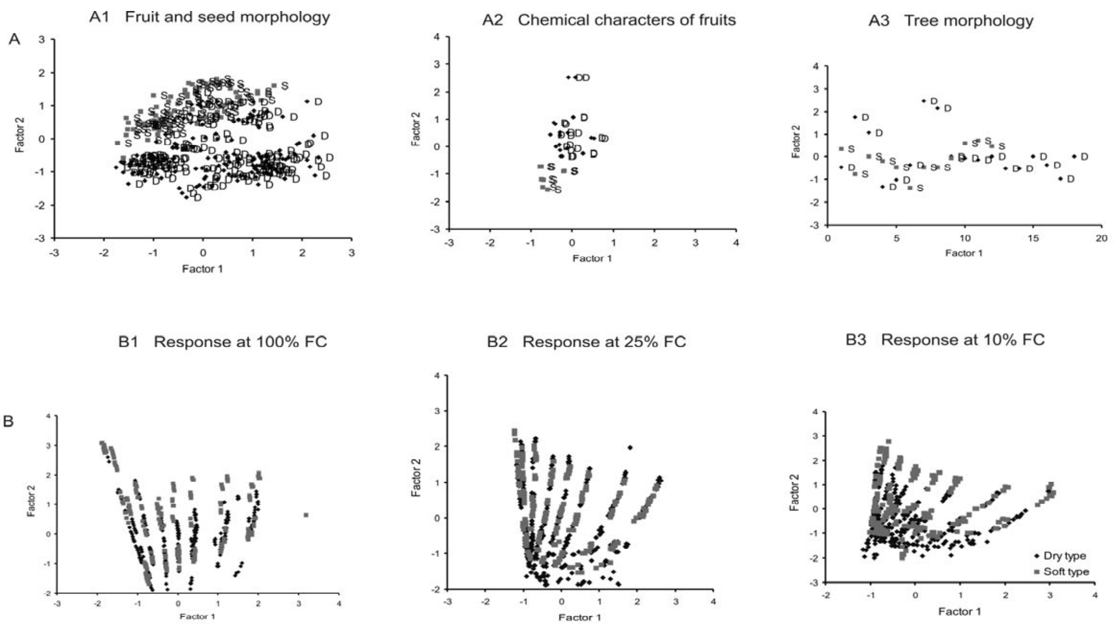

Figure 5. Comparable dry and soft types differentiation due to A. morphological traits under normal field irrigation conditions and B. differentiation due to physiological traits induced by water stress under greenhouse conditions.

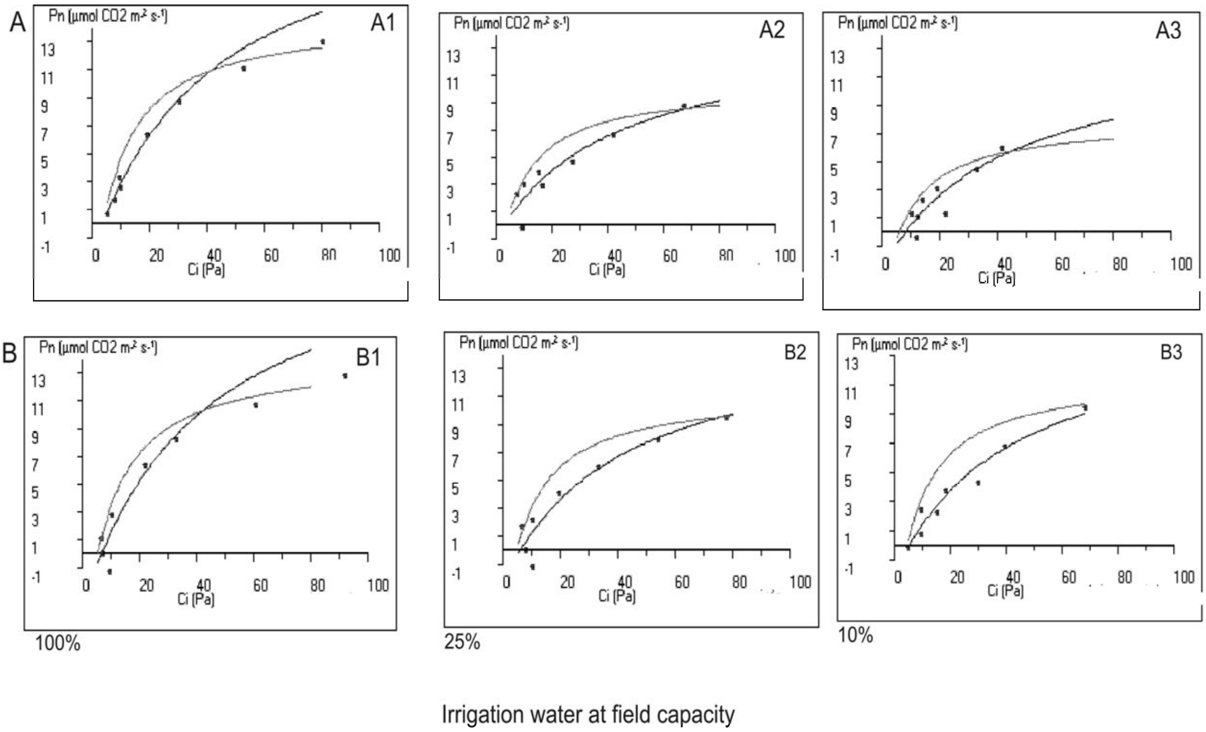

Figure 6. Pn (Photosynthesis in µmol CO_2 m^{-2} s^{-1}) / Ci (in Pa; Pascal) response curve patterns of A. dry and B. soft phenotypes. Each panel contains three different water levels, from left to right: 100%, 25% and 10% of field capacity.

4. CONCLUSIONS

High genetic (**Paper 1 and 2)** as well as compositional and morphological (**Paper 3)** diversity seems to be a general characteristic feature of date palm germplasm as revealed in this study and in other research including different production areas. This diversity constitutes a wealth that needs to be well-recognized and maintained for evolutionary responses in a changing environment and for breeding purposes to improve the agronomical and commercial characters of date palm. Specifically for Sudan and Morocco, based on this study, we can conclude that the major features of date palm cultivars (**Paper 1)** include the complete lack of clustering and the absence of cultivars representing specific clones, which is in contrast to previous assumptions, especially for Sudan. It follows that the cultivar nomenclature and classification in Sudan still remain to be based on fruit characters, including morphological, physical and chemical traits, and we are still unable to answer the question of how to confirm the genetic identity and true to typeness of each cultivar, especially when studying tissue culture derived date palms. The present identity determination is possible only after first flowering and fruiting stages at the age of about 5-7 years. World-wide date palm genomes need further investigations and also the identification of proper markers that may assist in identifying the economically and agronomically important cultivars.

When populations rather than just individual cultivars were genotyped (**Paper 2)**, it was evident that the genetic structure of date palms is governed by multiple factors. Three main factors, which indicate the involvement of a natural mechanism, were apparent as affecting the genetic differentiation of the trees in form of populations and/or cultivar groups. These factors include: the geographic isolation by distance, the biological nature of the tree and the environmental characters of the specific region, e.g., the degree of aridity is represented strongly in fruit characters and slightly in populations differentiation. Although, the history of cultivation and cultural practices are difficult to trace, they may constitute a strong effect in the structuring of date palms. The complex exchange processes of date palm germplasm throughout the world following cultivar and/or pollen grain selection, along with the random distribution of dispersal agents point out that, among other factors, the human impact possibly creates the highest influence on structuring date palm genome worldwide.

It is well known that date palm culture is associated with dry lands, and date palm is a drought-tolerant tree with a characteristic xerophytic feature of a deep network of root systems. Yet, irrigation water is needed for optimum yield. Thus far, the majority of growth stress studies on trees have been conducted on woody plant species, which are dicotyledonous trees. In this study, in **Paper 4,** date palm plants showed interesting responses to water stress: The studied cultivars and phenotypes showed specific effect and/or interaction effects due to water availability on number of pinnae, plant height and root dry weight. These traits can be considered for further studies related to drought adaptation in date palm. Soft and dry phenotypes have different specific morphological as well as physiological adjustments in response to different levels of water availability, while the dry types apparently were more sensitive to water stress. Generally the studied date palm plants showed high fixation capacity to photosynthetic CO_2 supply with interaction effect to water availability, which may considered as advantageous when coping with stresses that may arise with climate change. The ratio of intercellular CO_2 to elevated 350 ppm in non-stressed plants was in the range of C_4 plant species (0.3-0.4), a result that needs to be investigated also under natural conditions. Although a possible reason of this Ci/ C_a ratio is the way plants respond to controlled conditions in the greenhouse, date palms may possess a specific strategy for gas exchange and photosynthetic metabolism, perhaps as it is a monocotyledonous desert tree. To our best knowledge, this is the first report (**Paper 4)**, on the mechanisms that enhance photosynthetic CO_2 supply and demand functions in date palm under water stress. We suggest that the monocot date palm tree can be used as a model tree to study photosynthetic responses to different types of stresses. Such investigations may also reveal different capacities among date palm plants to cope with varying environmental conditions, including more drastic drought, salinity and temperature induced by changing climate.

Differentiation of the two phenotypes in response to water stress (**Paper 4**) is consistent with detected morphological, compositional (**Paper 3**) and genetic (**Paper 2**) differentiation. The genetic differentiation may cover specific traits that contribute to adaptive mechanisms in each phenotype. Further investigations to identify specific traits for drought adaptation as well as possible drought responsive genes are needed. Number of pinnae, plant height, adjustment of root/shoot characters, induced leaf senescence, stomatal conductance and the adjustment of the photosynthetic CO_2 supply and demand functions - apparent in the J_{max}/V_{max} ratio - were among the main traits that contribute to adaptation to drought and may, on the other hand, contribute to the ecological distribution of soft and dry types of dates.

Although, a large amount of diversity exists among date palm germplasm, the use of this wide range of choices for selection and adaptation of date palm cultivars is constrained by the difficulties of screening and documenting all existing cultivars. These difficulties can be minimized by the use of a combination of different methods for the analysis of date palm cultivars utilized in this study, effective when exploring the overall role of different characters in grouping date palm cultivars. Apparently, a number of markers can be applied as group descriptors according to specific objectives. Different marker systems and their combinations may have considerable value when screening wide date palm collections for the preliminary characterization of germplasm, e.g. considering the grouping of soft and dry types of dates. Such analyses may also provide useful information about geographic distribution and dispersal, traits for quality assessment and traits for breeding programmes.

5. ACKNOWLEDGEMENTS

"In the name of God, Most Gracious, Most Merciful, Praise be to God".

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